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8-Methoxypsoralen induced mutations are highly targeted at crosslinkable sites of photoaddition on the non-transcribed strand of a mammalian chromosomal gene

E.Sage, E.A.Drobetsky and E.Moustacchi

CNRS URA 1292, Institut Curie-Section de Biologie, 26 rue d'Ulm, 75231 Paris Cedex 05, France

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We have determined the mutational specificity of 8-methoxypsoralen photoaddition at the endogenous adenine phosphoribosyltransferase gene of Chinese hamster ovary cells hemizygous for this locus. In addition, the distribution of 8-methoxypsoralen photoadducts was resolved *in vitro* at the DNA sequence level, and compared with the observed site specificity for mutation. Among 27 mutants characterized, all were single base changes at AT base pairs: 16 A:T → T:A, six A:T → C:G, four A:T → G:C and one −T frameshift. All these vents were targeted to potential sites of photoaddition. The vast majority of these sites were also detectable *in vitro*, suggesting that 8-methoxypsoralen plus UVA-induced mutational hotspots may be damage hotspots. Furthermore 26/27 mutations occurred at crosslinkable 5′TpA sites, supporting the notion that 8-methoxypsoralen biadducts rather than monoadducts are major premutagenic lesions in mammalian cells. Since 90% of our mutation collection could have resulted from damage on the non-transcribed strand, it appears that photoadducted thymine residues on the transcribed strand of the adenine phosphoribosyltransferase gene may be preferentially repaired. We therefore suggest a model for mutagenesis, induced by psoralen biadducts, based on the preferential incision of biadducts followed by translesion synthesis past modified T bases persisting on the non-transcribed strand.

Key words: APRT locus/DNA crosslinks/furocoumarins/mutagenesis/preferential repair

Introduction

A powerful approach for understanding cellular responses to DNA damaged by a particular agent is to determine the specificity of both mutation and damage within a target gene. Such combined analyses, previously performed in bacteria (Brash and Haseltine, 1982; Koehler *et al.*, 1991), as well as in shuttle vectors propagated in mammalian cells (Brash *et al.*, 1987; Roilides *et al.*, 1988; Sage and Bredberg, 1991; Yang *et al.*, 1988), have given insight into the role of premutagenic lesions, DNA context and DNA repair processes in mutagenesis.

The utility of this strategy is predicated on the clear demonstration that some agents produce a characteristic pattern of mutations, or 'mutational fingerprint'. Thus, mutation spectra obtained in cultured cells have been useful in understanding the origins of multistep carcinogenesis in man, and can permit the identification of the causative carcinogen(s). For example, the involvement of UV-induced dipyrimidine photoproducts in p53 tumour suppressor gene mutations from squamous cell carcinoma of the skin has been clearly demonstrated (Brash *et al.*, 1991). Similarly, mutational hotspots of the p53 gene from hepatic tumours in African patients have been attributed to the N7−guanine adduct of aflatoxin B1 (Hollstein *et al.*, 1991).

Furocoumarins of natural and synthetic origin are becoming increasingly important as photochemotherapeutic agents, and are widespread components of the diet at potentially genotoxic levels (IARC, 1986). It is therefore important to understand the mechanisms whereby these compounds realize their mutagenic/carcinogenic potential. The genotoxicity of psoralen derivatives, a class of furocoumarin, has been attributed to their ability to covalently bind DNA upon UVA irradiation. Briefly, these compounds intercalate into DNA and undergo photocycloaddition with pyrimidines (mainly thymine) in a sequence specific manner (Gamper *et al.*, 1984; Cimino *et al.*, 1985; Sage and Moustacchi, 1987) to form both monoadducts and biadducts, the latter yielding interstrand crosslinks. 5′TpA site is the preferred target in the formation of monoadducts, and the exclusive one for interstrand crosslinks (Gamper *et al.*, 1984; Sage and Moustacchi, 1987). Evidence derived from a number of different systems suggests that biadducts rather than monoadducts are responsible for the genotoxic effects of psoralens (for review see Averbeck *et al.*, 1991).

Here we have investigated the mutational specificity of the bifunctional psoralen derivative 8-methoxypsoralen (8-MOP) plus UVA at the endogenous adenine phosphoribosyltransferase (*APRT*) locus in Chinese hamster ovary (CHO) cells, and compared this with the spectrum of damage induced *in vitro* in the same gene. The present work is the first example of such a combined analysis at an endogenous locus in mammalian cells.

Results

Distribution of 8-MOP photoadducts at the APRT locus

To investigate the relationship between furocoumarin-induced damage and mutation, the distribution of photoadducts produced *in vitro* by 8-MOP in the coding sequence of the CHO *APRT* gene was first determined. Sites of photoaddition were revealed on sequencing gels as termination sites for the 3′−5′ exonuclease of T4 DNA polymerase. The activity of this enzyme is blocked by psoralen biadducts, as well as by monoadducts (Sage and Moustacchi, 1987). As exemplified for one exon in Figure 1, 8-MOP photoadducts occurred in majority at 5′TpA sequences and were not evenly distributed at such sites. Indeed, in the whole *APRT* gene 8-MOP photoadducts were mainly distributed among so-called 'strong sites' (Sage and Moustacchi, 1987; Boyer *et al.*, 1988), i.e. AT rich

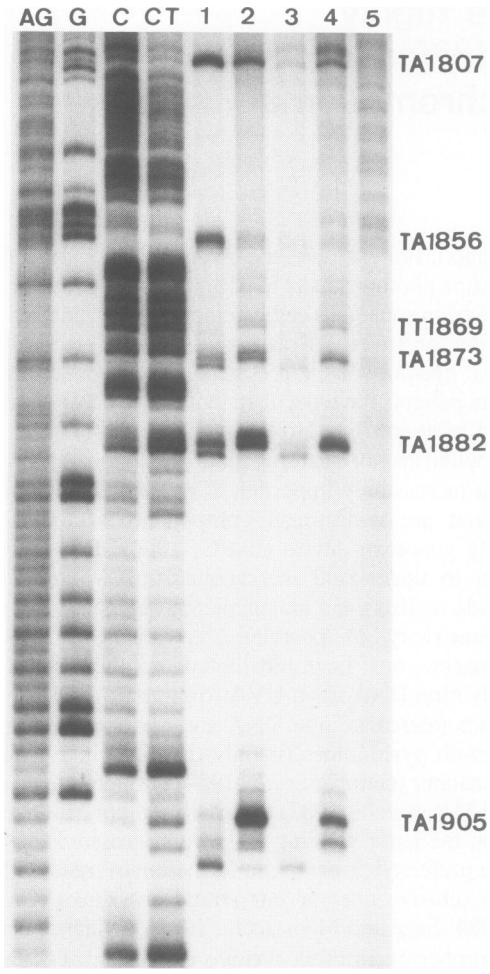

Fig. 1. Sites of 8-MOP photoaddition induced *in vitro* in exon 5 *APRT* DNA. A DNA fragment containing exon 5 was labelled at the 5′ end of the strand corresponding to the transcribed strand. Labelled DNA was incubated in the presence of 8-MOP and UVA irradiated at fluences of 24 kJ/m^2 (lanes 1, 2 and 5) or 6 kJ/m^2 (lanes 3 and 4) and digested with T4 DNA polymerase 3′−5′ exonuclease (lanes 1−4) or undigested (lane 5). In lanes 1 and 3 crosslinks were photoreversed, consequently these lanes reveal both mono- plus bi-adducts, whereas lanes 2 and 4 show monoadducts only (for more details see Sage and Moustacchi, 1987). Note that less radioactivity was loaded in lanes 1 and 3.

sequences, and formed preferentially at crosslinkable 5′TpA sites (Figure 2). Moreover, the repeated (A-T)$_n$ sequences in exons 2 and 5 were hotspots for photoaddition, in agreement with our previous data (Sage and Moustacchi, 1987). Unexpectedly, photoadducts were not recovered at T runs. This is in contrast to the *lacI* gene of *Escherichia coli*, where such sites were significantly more reactive (Sage and Moustacchi, 1987). Also, some 5′TpA sites either led only to monoaddition or did not appear to photoreact (Figure 2). In fact most of these sites fall into a category with decreased crosslinking ability, i.e. 5′TpA dinucleotides with a purine on the 3′ side or surrounded by runs of purine residues within two base pairs (Esposito *et al.*, 1988). Moreover, the rate of crosslinking depends not only on neighbouring bases, but on long range sequence context as well (Esposito *et al.*, 1988). In this respect, the richness of the *APRT* gene in GC blocks, may create an unfavourable environment for psoralen photobinding.

Specificity of 8-MOP-photoinduced mutations at the APRT locus

The nature of 8-MOP-photoinduced mutations in the endogenous *APRT* gene was determined at the DNA sequence level. A collection of 27 independent 8-azaadenine resistant (8-AA′) clones induced by 8-MOP plus UVA was obtained from Chinese hamster D422 cells hemizygous for the autosomal *APRT* gene (Nalbantoglu *et al.*, 1983). The treatment applied (5 μM 8-MOP and 3 kJ/m^{-2} UVA) resulted in a survival level of 12%, and a mutational induction of ~30-fold above background.

None of the 27 mutants analysed exhibited any alteration in the size of the amplified *APRT*-containing DNA fragment, demonstrating that they did not result from major rearrangements or large deletion events. Indeed, the exclusive presence of point mutations was confirmed by sequencing the entire coding region (540 bp) of each mutant (Figure 2 and Table I). This analysis revealed 26 single base substitutions and a single −T frameshift, all of which occurred at T residues. Although T:A → A:T transversions predominated (59%), T:A → G:C transversions (22%) and T:A → C:G transitions (15%) were also recovered.

Most of the mutations were recovered at the first or second position of amino acid codons; an additional two resulted from modification of a 5′ splice junction at position 1429. The mutational hotspot at position 212 involved the third base of an aspartic acid codon. Since T to C transitions would not lead to an amino acid change it could be slightly under-represented.

Correlation between damage and mutation spectra

Examination of the results presented in Figure 2 reveals that every mutation in our collection is targeted at a potential site of photoaddition, the vast majority at crosslinkable 5′TpA sites. In addition, almost all the mutations arose at sites where photoadducts were actually detected *in vitro*. Indeed, no mutations were recovered at 5′TpA sites where no damage was observed (bars in Figure 2). Photodamage at the mutated splice site (position 1429) was not determined since only exons were analysed. Also, no mutation was observed where 8-MOP monoadducts rarely form, such as at C residues. Only one mutation arose at a 5′ApT site (position 310), a non-crosslinkable site which nevertheless, can lead to the formation of monoadducts, at least *in vitro* (Gamper *et al.*, 1984; Sage and Moustacchi, 1987). However, under our experimental conditions monoadducts were not detected at this position.

Notably, 8-MOP-induced mutations are not distributed randomly among potential sites of photodamage. Thirteen base changes are observed at only two sites. One of these two mutational hotspots is also a hotspot for photoaddition (position 212), the other being relatively less photoreactive (position 1419).

Strand specificity in the induction of mutation

Among the 27 mutants analysed, 24 arose from an adducted T located on the non-transcribed strand (as deduced from the amino acid sequence of the APRT polypeptide). This suggests that psoralen photoadducts located on the transcribed strand are preferentially incised.

Discussion

Using the endogenous CHO *APRT* gene as target, we have determined the mutational specificity of 8-MOP plus UVA. This has been compared with the damage distribution produced by the same agent *in vitro*. The mutation spectrum is comprised exclusively of single base changes which result in amino acid substitutions, or alteration of a splice site. It is striking that all mutations were recovered at potential sites of photoaddition. Indeed, all except one occurred at T residues within crosslinkable sites, 5'TpA. Two mutational hotspots (at positions 212 and 1419) were observed, one of which is also a hotspot for photoaddition (position 212). These observations demonstrate that mutagenesis by 8-MOP plus UVA at an endogenous locus in a rodent cell line is highly targeted at photoadducts, and may be due to biadducts.

In the *APRT* gene, 100% of the 8-MOP-induced mutants were point mutations at A:T base pairs: T:A → A:T transversions predominated, although T:A → G:C and T:A → C:G events were also represented. In other bacterial and mammalian systems, psoralen derivatives also induce mainly point mutations primarily at A:T base pairs, although the T:A → A:T transversions may not be the preferred event (Piette *et al.*, 1985; Miller and Eisenstadt, 1987; Yatagai *et al.*, 1987). In the *lacI* gene of *E.coli*, 65% of the 8-MOP-induced mutations occurred at A:T base pairs (Yatagai *et al.*, 1987). In the same gene, hotspots of mutation induced by angelicin occur at T residues within a repeated TA context (Miller and Eisenstadt, 1987), which are strong sites for angelicin photobinding (Boyer *et al.*, 1988; Miolo *et al.*, 1989). In addition, all seven *HPRT*$^-$ 4'-hydroxymethyl-4,5', 8-trimethylpsoralen (HMT)-induced base substitution mutations isolated from mouse cells were recovered at A:T base pairs, four within an ATA/TAT context (Piette, 1992).

In sharp contrast to the *APRT* locus, a significant proportion of mutations in bacterial genes, as well as in an extrachromosomal shuttle vector propagated in mammalian cells (Bredberg and Nachmansson, 1987; Miller and Eisenstadt, 1987; Yatagai *et al.*, 1987), were recovered at G:C base pairs, where psoralen photoadducts are extremely rare. This may reflect differences in replisome and/or repairosome structure and function with respect to processing of psoralen-induced damage in endogenous mammalian genes versus shuttle vectors or bacteria. In addition, unlike the case for *lacI* of *E.coli* (Yatagai and Glickman, 1986) and *HPRT*$^-$ in human cells (Papadopoulo *et al.*, 1990), deletions induced by psoralen are not recovered either at the CHO *APRT* locus, or at the mouse *HPRT* locus (Piette, 1992). This may result from differences in the kinetics and efficiency of excision repair (Ganesan *et al.*, 1983; Yagi *et al.*, 1984) and/or from the existence of essential genes within the region of hemizygosity of the *APRT* gene which render large deletions lethal.

The relative mutagenicity of mono- versus biadducts in CHO cells

An important conclusion of our work is that biadducts rather than monoadducts are likely to be the major psoralen-induced premutagenic lesion in mammalian cells. Indeed, this is supported by the almost exclusive occurrence of *APRT*$^-$ mutations at crosslinkable 5'TpA sites within AT-rich sequences, rather than at potential sites of monoadducts (e.g. 5'ApT). Also, the damage hotspot at position 214, where only monoadducts can possibly form, was not recovered as a site of mutation (see below). Evidence from other systems also indicate a pre-eminent role for biadducts in mutagenesis. They are unquestionably more mutagenic than monoadducts in yeast (Cassier *et al.*, 1984), as well as in a shuttle vector propagated in mammalian cells (Bredberg and Nachmansson, 1987). Also, in bacteria, mutagenesis appears to depend on the incision of psoralen crosslinks, rather than monoadducts (Sladek *et al.*, 1989). Furthermore, in mammalian cells, monoadducts are more slowly excised than biadducts from bulk DNA as well as from actively transcribed genes (Vos and Hanawalt, 1987; Wauthier *et al.*, 1990; Vos and Wauthier, 1991; Islas *et al.*, 1991), and may persist in cells (Vos and Hanawalt, 1987). Finally, monoadducts can be bypassed by DNA polymerase both *in vitro* and *in vivo* (Piette and Hearst, 1983; Chanet *et al.*, 1985; Piette *et al.*, 1985) probably in an error-free manner (Vos and Hanawalt, 1987).

8-MOP-photoinduced mutations are highly targeted at sites of photoaddition

It is clear that 8-MOP-induced mutations in our system are highly targeted at strong sites of photoaddition. The correlation between damage and mutation frequencies at individual sites in the *APRT* gene appears to be much better for 8-MOP plus UVA, than for 254 nm UV light (E.A.Drobetsky and E.Sage, unpublished). Moreover, since 8-MOP-induced mutations arise at sites where photoadducts are also detectable *in vitro*, it appears that mutations may be preferentially recovered at damage hotspots. However, DNA sequence context effects, and/or the relative mutagenicity of mono-versus biadducts may also be involved. For example, the former argument could explain the presence of only two mutations at the photoadduct hotspot T-1904, where mono- as well as biadducts can form. Alternatively, at the damage hotspot in exon 2 (5'ATAT), no mutation was found at the second T (position 214), even though a T → A transversion has been recovered here after irradiation with 254 nm UV light (Drobetsky *et al.*, 1987). The first T (position 212) is both hot for mutation and crosslinkable, whereas only monoadducts can form at position 214. In addition, among three 5'TpA sites which were not photoadducted *in vitro*, none were mutated (Figure 2), even though sequence alterations here would have resulted in amino acid changes. We also note that no mutations were recovered at position T-1855 (a strong site for photoaddition) since any base substitution at this site would not yield an amino acid change.

Preferential incision of 8-MOP photoadducted thymine located on transcribed strand

There is now evidence that DNA repair may be directly coupled to the transcriptional machinery (Hanawalt, 1991). It has also been demonstrated that psoralen photoadducts in mammalian cells are preferentially removed from actively transcribed genes, compared with the genome overall (Zolan *et al.*, 1984; Islas *et al.*, 1991; Vos and Wauthier, 1991). The vast majority (90%) of 8-MOP-induced mutations in our mutant collection could have resulted from a damaged T residue located on the non-transcribed strand. This strongly suggests that photoadducts on the transcribed strand of the *APRT* locus are preferentially repaired.

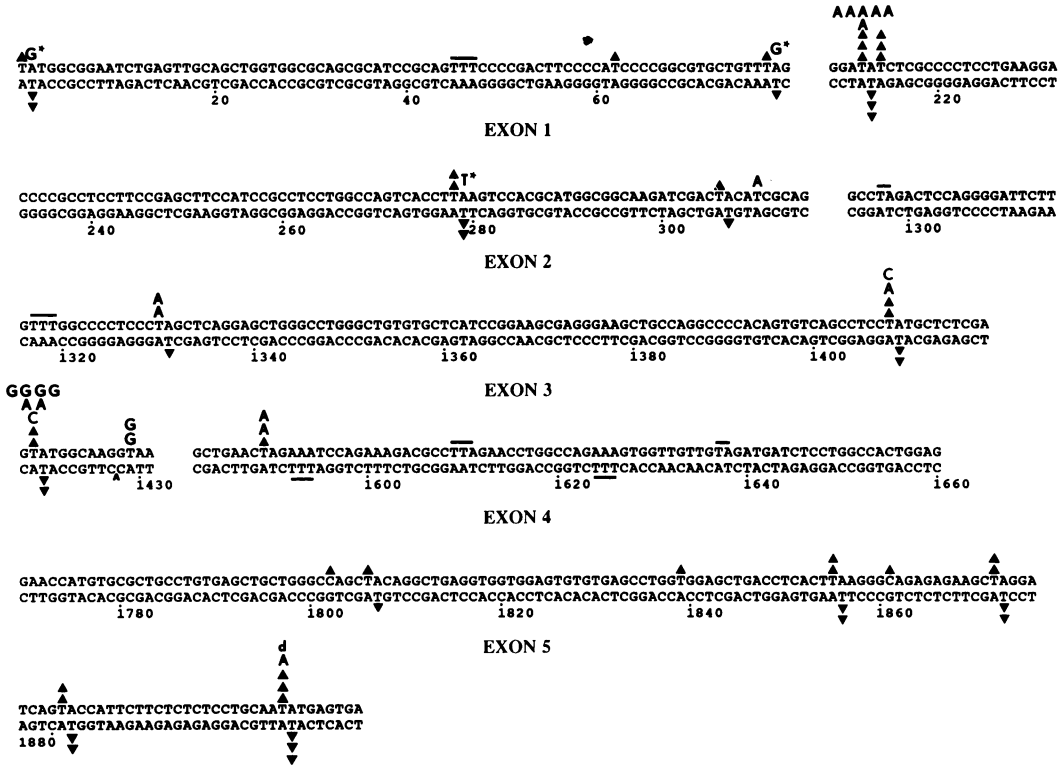

Fig. 2. Distribution of 8-MOP-induced photoadditions and mutations in the coding region of the endogenous *APRT* gene of CHO cells. Point mutations are indicated above the altered base in the upper (non-transcribed) strand. The stars at positions 1, 79 and 279 indicate that mutations arose from an adducted T on the transcribed strand. Sites and extent of photoaddition (mono- plus biaddition) are shown as black triangles. Bars above the sequence represent possible sites of photoaddition where no photoadducts were detected. Exon 3 ends at position 1427.

Table I. 8-MOP-photoinduced mutations at the APRT locus in CHO cells

Mutants	Position	Type of mutation	Amino acid change	Surrounding sequence (coding strand)	Strand with affected Py
D8-22a	1	TA → CG	Met → Val	GGCT A TGGC	t
D8-10a	79	TA → CG	Arg → Gly	GTTT A GGTG	t
D8-2a	212	TA → AT	Asp → Glu	GGGA T ATCT	nt
D8-7a	212	TA → AT	Asp → Glu	GGGA T ATCT	nt
D8-9b	212	TA → AT	Asp → Glu	GGGA T ATCT	nt
D8-11a	212	TA → AT	Asp → Glu	GGGA T ATCT	nt
D8-17a	212	TA → AT	Asp → Glu	GGGA T ATCT	nt
D8-23a	212	TA → AT	Asp → Glu	GGGA T ATCT	nt
D8-227b	279	TA → AT	Lys → stop	CCTT A AGTC	t
D8-3b	310	TA → AT	Ile → Asn	TACA T CGCA	nt
D8-3a	1330	TA → AT	Leu → Gln	TCCC T AGCT	nt
D8-13a	1330	TA → AT	Leu → Gln	TCCC T AGCT	nt
D8-1a	1407	TA → AT	Tyr → Asn	CTCC T ATGC	nt
D8-6a	1407	TA → CG	Tyr → His	CTCC T ATGC	nt
D8-8a	1419	TA → AT	Tyr → Asn	CGAG T ATGG	nt
D8-17b	1419	TA → CG	Tyr → His	CGAG T ATGG	nt
D8-18a	1419	TA → AT	Tyr → Asn	CGAG T ATGG	nt
D8-19a	1419	TA → GC	Tyr → Asp	CGAG T ATGG	nt
D8-24a	1419	TA → GC	Tyr → Asp	CGAG T ATGG	nt
D8-25a	1419	TA → GC	Tyr → Asp	CGAG T ATGG	nt
D8-26a	1419	TA → GC	Tyr → Asp	CGAG T ATGG	nt
D8-5a	1429	TA → GC	splice	AAGG T AAGC	nt
D8-20a	1429	TA → GC	splice	AAGG T AAGC	nt
D8-12b	1589	TA → AT	Leu → Gln	GAAC A AGAA	nt
D8-14a	1589	TA → AT	Leu → Gln	GAAC T AGAA	nt
D8-16a	1904	TA → AT	Tyr → Asn	GCAA T ATGA	nt
D8-15a	1904	T deletion		GCAA T ATGA	nt

The mechanisms of psoralen biadduct repair and mutagenesis in mammalian cells has not yet been elucidated (Smith, 1988). These processes are much better understood in *E.coli*. The repair of biadducts has been well characterized *in vitro* and *in vivo*, and involves two steps in excision separated by a recombinational event (Cole *et al.*, 1978; Van Houten *et al.*, 1986). It has also been demonstrated that incision by UvrABC excinuclease is a step for mutagenesis by psoralen crosslinks (Yatagai and Glickman, 1986; Yatagai *et al.*, 1987; Sladek *et al.*, 1989). Furthermore, the presence of DNA homologous to a target gene treated with psoralen plus UVA reduces the yield of point mutations, presumably by increasing the opportunity for error-free recombinational repair of biadducts (Sladek *et al.*, 1989).

In mammalian cells, it may be reasonable to expect a comparable pathway. In human cells, two endonucleases have been implicated in the excision of psoralen photoadducts. One of these is more specific for biadducts, while the other excises monoadducts as well as 254 nm UV-induced lesions (Lambert *et al.*, 1988). Furthermore, a recent description of incision of pyrimidine dimers by a human nuclease showed the release of a 27−29 nucleotide oligomer containing the dimer (Huang *et al.*, 1992). In addition, Reardon *et al.* (1991) have presented evidence for gap filling during excision repair of psoralen-induced biadducts, using a cell-free extract system. Our data is consistent with the preferential incision of biadducts on each side of the crosslinked thymine located on the transcribed strand. Since the adducted T on the non-transcribed strand would be incised more slowly, a T intermediate on this strand containing a bound psoralen molecule attached to an oligonucleotide, would be favoured. This partly incised crosslink could then act as a substrate for competing mutagenic and recombinational pathways (Figure 3). It should be noted that the CHO strain used in our study is hemizygous for the *APRT* gene and does not harbour *APRT* pseudogenes. It is thus interesting that our system may have allowed us to identify one pathway for mutagenesis by furocoumarin photoadducts, since the repair of an incised crosslink may not proceed through a recombinational pathway. We therefore suggest that mutational events in CHO cells occur by translesion synthesis across the modified T during repair synthesis, or DNA replication (Figure 3). Since differences in excision repair capacity exist between human and rodent cells, it therefore remains to be investigated whether this model for psoralen mutagenesis can also be applied to the human situation.

A model based on a mechanism of transient misalignment (Kunkel, 1990) has recently been presented to explain replication-dependent mutagenesis by psoralen monoadducts (Strazewski, 1991). We believe that this hypothesis can also be invoked to explain mutagenesis by incised crosslinks (Figure 3). The modified T generated as described above may transiently loop out when encountered by the polymerase. This would allow incorporation of the next nucleotide into the growing chain. The looped out modified T could then flip back into the helix, thus realigning the strands and permitting further elongation. The mispair formed would yield a base substitution templated by the base immediately 5′ to the modified T. Such a model could explain 63% of the base changes identified in our study.

In conclusion, we have demonstrated that psoralen-induced mutations are highly targeted at crosslinkable sites at an endogenous locus in CHO cells, and are likely to result from biadducts. The excellent correlation between the damage distribution determined *in vitro*, and the mutation spectrum obtained *in vivo* indicates that the sites and frequency of photoadducts formed in chromatin are likely to be very similar to what is observed *in vitro* for naked DNA. This finding increases the credibility of *in vitro* studies of damage distribution.

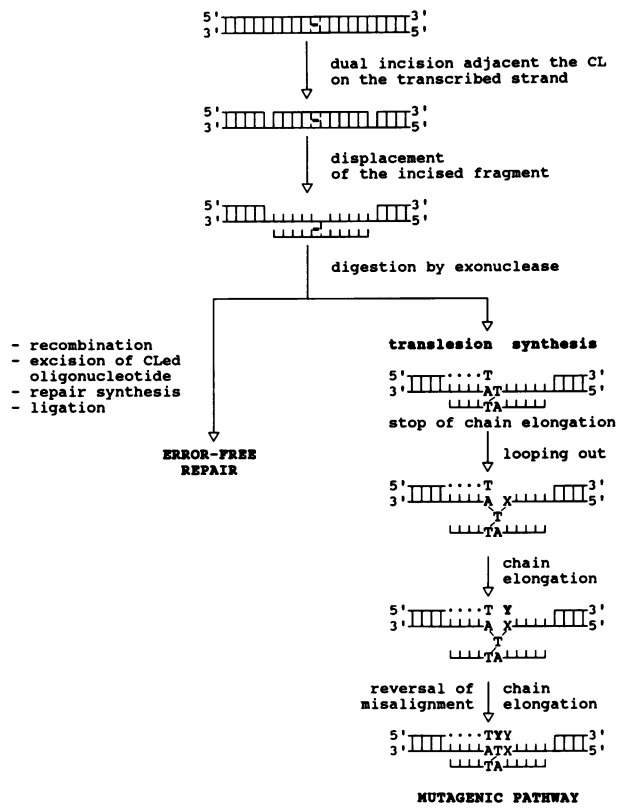

Fig. 3. Processing of psoralen-photoinduced crosslinks in rodent cells. This model combines schemes proposed by Van Houten *et al.* (1986) (error-free repair), and by Kunkel (1990) and Strazewski (1991) (translesion synthesis by transient misalignment). In the mutagenic pathway, after replication, the following mutagenic events are expected: when X=A=>T→A; X=G=>T→G; X=C=>T→C. Mutation does not occur when X=T=>T→T. CL, crosslink.

Materials and methods

Cells

The Chinese hamster ovary (CHO) strain D422 (Nalbantoglu *et al.*, 1983) used in this study is an *APRT* hemizygote originally derived from a CHO line auxotrophic for proline. Cells were routinely maintained in growth medium consisting of alpha minimal essential medium supplemented with 5% fetal calf serum (Gibco).

Mutagenic treatment

Growth medium from 100 mm dishes containing 10^7 exponentially growing cells was aspirated, and replaced with 5 ml phosphate buffered saline (PBS) containing 5 μM 8-MOP. The cells were incubated for 10 min in the dark at 37°C and then irradiated with a HPW 125 Philips lamp emitting mainly at 365 nm, at a fluence rate of 10 J/m^2/s. The fluence received was 3 kJ/m^2. As controls some cells were either untreated, exposed to radiation alone or to 8-MOP alone. After irradiation, unreacted 8-MOP was removed by washing with PBS, and cells from each plate were immediately trypsinized and divided among ten 100 mm plates. After a 5 day phenotypic expression period, *APRT*$^-$ mutants were selected by seeding two replicates of 5 × 10^5 cells from each culture on 100 mm dishes in growth medium containing 0.4 mM 8-azaadenine (8-AA). To ensure that *APRT*$^-$ mutants were

independent, only one 8-AA^r clone was collected from each plate for further characterization.

PCR amplification and direct sequencing of mutant APRT DNA

A 21 kbp DNA fragment, spanning positions −30 and +2107 of the published *APRT* gene sequence (de Boer *et al.*, 1989) and including the entire coding region plus introns, was amplified by the polymerase chain reaction (PCR) using a nested priming approach. A typical 100 μl reaction containing 60 mM KCl, 15 mM Tris−HCl pH 8.3, 2.7 mM $MgCl_2$, 200 μM each dNTP, 0.1 μM of each primer as described previously (Drobetsky and Glickman, 1990), and 2.5 units Taq DNA polymerase (Bethesda Research Laboratories, BRL). Primary reactions, containing 1 μg genomic DNA as substrate, were performed (30 cycles) at 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, after an initial denaturation step at 94°C for 5 min. One μl of this reaction was then used as substrate for a nested reaction, which was necessary to obtain a sufficient yield of *APRT* DNA for subsequent analysis.

Prior to sequencing, amplified mutant *APRT* DNA was purified by electrophoresis through low melting point agarose (BRL). The entire coding region of each mutant was determined using either a protocol for direct double stranded DNA sequencing of PCR products with Sequenase version 2.0 (United States Biochemical) in the presence of dimethylsulfoxide (Winship, 1989), or the cycle sequencing protocol using Taq DNA polymerase (BRL).

Site-specific determination of 8-MOP photoadducts

The plasmid pRVA3 (Drobetsky *et al.*, 1989), which carries the CHO *APRT* cDNA, was used as substrate for amplification of three DNA fragments containing exons 1 and 2, 3 and 4, and 5, respectively. These fragments were labelled on the 5′ end of the one or the other strand during PCR, using a single ^{32}P-end labelled primer. Labelled amplified products were purified on 8% non-denaturing preparative polyacrylamide gels. DNA fragments were then exposed to 50 μM 8-MOP, and UVA-irradiated at a fluence of 6 (Figures 1 and 2) or 24 kJ/m^2 (Figure 1). We note that although the 8-MOP concentration utilized here was 10-fold higher than that used to treat cells, the photoadduct distribution is similar in both cases (E.Sage, unpublished observation). Sites of photoaddition at the DNA sequence level were revealed as previously described (Sage and Moustacchi, 1987). Briefly, photoadducted DNA was digested with 4.5 units of T4 DNA polymerase 3′−5′ exonuclease, in the absence of dNTPs, and analysed alongside a Maxam and Gilbert sequencing ladder, after photoreversion of interstrand crosslinks by a fluence of 6 kJ/m^2 of UVC radiation. The yield of photoadducts (mono- plus biadducts) at each site was estimated after counting the radioactivity in gel slices corresponding to bands on autoradiograms. The ratio of monoadducts to biadducts at individual sites was not determined since the conversion of monoadducts into biadducts strongly depends on the UVA fluence and it will not reflect the *in vivo* situation.

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